Effect of leaf age and position on light-saturated CO_2 assimilation rate, photosynthetic capacity, and stomatal conductance in rubber trees

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Abstract

Shoots of the tropical latex-producing tree *Hevea brasiliensis* (rubber tree) grow according to a periodic pattern, producing four to five whorls of leaves per year. All leaves in the same whorl were considered to be in the same leaf-age class, in order to assess the evolution of photosynthesis with leaf age in three clones of rubber trees, in a plantation in eastern Thailand. Light-saturated CO_2 assimilation rate (A_{max}) decreased more with leaf age than did photosynthetic capacity (maximal rate of carboxylation, V_{cmax}, and maximum rate of electron transport, J_{max}), which was estimated by fitting a biochemical photosynthesis model to the CO_2-response curves. Nitrogen-use efficiency (A_{max}/N_a, N_a is nitrogen content per leaf area) decreased also with leaf age, whereas J_{max} and V_{cmax} did not correlate with N_a. Although measurements were performed during the rainy season, the leaf gas exchange parameter that showed the best correlation with A_{max} was stomatal conductance (g_s). An asymptotic function was fitted to the A_{max}-g_s relationship, with $R^2 = 0.85$. A_{max}, V_{cmax}, J_{max} and g_s varied more among different whorls in the same clone than among different clones in the same whorl. We concluded that leaf whorl was an appropriate parameter to characterize leaves for the purpose of modelling canopy photosynthesis in field-grown rubber trees, and that stomatal conductance was the most important variable explaining changes in A_{max} with leaf age in rubber trees.

Additional keywords: *Hevea brasiliensis*; leaf whorl; maximum rate of carboxylation; maximum rate of electron transport; nitrogen; photosynthesis; rubber clone.

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Abbreviations: A – net CO_2 assimilation rate or net photosynthetic rate; A_c – net assimilation rate limited by activity of Rubisco; A_j – net assimilation rate limited by RuBP concentration; A_{max} – light-saturated net CO_2 assimilation rate; C_c – CO_2 partial pressure at the carboxylation sites; C_i – intercellular leaf CO_2 partial pressure; DM – dry mass; E_a – activation energy; g_i – internal conductance; g_s – stomatal conductance; J_{max} – maximum rate of electron transport (or RuBP regeneration); K_c – Michaelis constant of Rubisco for carboxylation [μmol mol^{-1}]; K_o – Michaelis constant of Rubisco for oxygenation [μmol mol^{-1}]; LMA – leaf mass per unit area; N_a – nitrogen content per unit leaf area; N_m – nitrogen content per unit leaf dry mass; O – partial pressure of O_2 in the intercellular air space [μmol mol^{-1}]; PPF – photosynthetic photon flux; R – gas constant (8.314 J K^{-1} mol^{-1}); R_D – dark respiration; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP – ribulose-1,5-bisphosphate; SPAD – SPAD index of chlorophyll content; T – leaf temperature; T_{ref} – reference temperature; V_{cmax} – maximum rate of RuBP carboxylation; VPD – vapour pressure deficit; Wn – whorl number; α – apparent quantum yield; Γ^* – CO_2 compensation point in the absence of dark respiration [μmol mol^{-1}].

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Introduction

Rubber is a major tree crop cultivated mainly under equatorial and monsoon climates (Indonesia, Malaysia, Southern Thailand). However, rubber tree plantations are currently expanding to non-traditional areas such as northern and northeastern Thailand, where they inevitably encounter a drier climate with a wider range in temperature. A better understanding of the physiological response of rubber trees to such changes in environment would help to predict potential production and the risk faced by farmers in these new areas. Among the relevant physiological traits, photosynthetic capacity of leaves is of first importance, as it determines carbon assimilation and therefore primary productivity. Following the pioneer studies by Samsuddin and Impens (1978b, 1978c) on clonal variations of photosynthesis in rubber trees, Samsuddin *et al*. (1987) showed that photosynthesis correlated with girth increment but not with latex yield. Photosynthesis traits were related to planting density (Samsuddin and Impens 1978a), adaptation to shade (Senevirathna *et al*. 2003), and tapping (Nugawela *et al*. 1990). However, to integrate the response of photosynthesis to environmental factors, modeling is required. At the leaf level, the model of Farquhar *et al*. (1980) is widely accepted. This model describes photosynthesis with two major parameters determining the biochemical capacities of the photosynthetic apparatus, V_{cmax} and J_{max}. These parameters vary widely between species (Wullschleger 1993, Dreyer *et al*. 2001, Medlyn *et al*. 2002). In rubber, Nataraja and Jacob (1999) described clonal differences in important photosynthetic traits such as compensation irradiance (CI) and apparent quantum yield of CO_2 (Φc), but V_{cmax} and J_{max} have not yet been estimated for different rubber clones. Moreover, Farquhar photosynthetic parameters have generally been assessed on young fully expanded leaves, ignoring leaf-age effect (Hikosaka *et al*. 1999, Yamori *et al*. 2005, Hikosaka *et al*. 2007). Establishing the effect of leaf age on photosynthetic characteristics of rubber leaves will provide basic information required to model canopy photosynthesis in field conditions. Samsuddin and Impens (1979b) and Antas Miguel *et al*. (2007) studied the effect of leaf age on photosynthesis in rubber trees, but without information on biochemical parameters that determine photosynthetic capacity. Beside photosynthetic capacity, CO_2 assimilation rate depends on the opening of leaf stomata, characterized by g_s. In temperate species, lower g_s has often been observed with leaf aging (Jordan *et al*. 1975, Field and Mooney 1986, Han *et al*. 2008). However few data are available on tropical species which have a completely different phenology. For instance, Kitajima *et al*. (2002) showed that g_s decreased with age (up to 90 days) in only one of the 2 pioneer tropical tree species they studied. The evolution of rubber g_s with leaf age has not been studied so far. Therefore, the aim of the present study was to assess the evolution with leaf age of photosynthesis and photosynthetic capacity as indicated by V_{cmax} and J_{max}, together with g_s, in three field-grown rubber clones. For this purpose, we took advantage of the specific periodic pattern of rubber shoot development, characterized by alternating periods of stem elongation, leaf development, and meristem rest (Hallé and Martin 1968, Combes and du Plessix 1974). This pattern gives the branches a verticillate or sub-verticillate arrangement. Each readily distinguishable portion of the axis grown between two rest periods is called a growth unit, and the set of leaves (six to fifteen) belonging to one growth unit is named a whorl. Therefore, whorl rank was chosen as a variable to represent classes of leaves differing in location along the branches and in age, which both influence leaf photosynthetic capacity (Kitajima *et al*. 2002, Xie and Luo 2003, Niinemets 2005, Han *et al*. 2008).

Materials and methods

Gas exchange measurements: All gas exchange measurements were made on attached leaves with a portable photosynthesis system (*LI-6400, LI-COR*, Lincoln, Nebraska, USA). Light was supplied with red-blue light emitting diodes (*6400-02B* LED light source). The CO_2 concentration of the reference air (360 µmol mol^{-1}) entering the leaf chamber was controlled with a CO_2 mixer. Air relative humidity and leaf temperature were maintained constant at ambient level during the measurement.

Experiment 1. Effect of leaf age on light-saturated CO_2 assimilation rate: This experiment aimed at characterizing the evolution of leaf gas exchange during the early stages when the leaves were still expanding and not mature. For practical reasons, these early stages would have been difficult to assess in the field experiment.

Plant material: Potted rubber trees (*Hevea brasiliensis* Muell. Arg.), clone RRIM 600, were grown in a nursery at Kasetsart University, Bangkok, Thailand in August 2005. All plants were well irrigated once per day. Experiment started when the trees were two months old.

Methodology: Light-saturated CO_2 assimilation rate or light-saturated photosynthetic rate (A_{max}) was measured on six intact newly emitted leaves of three trees. Stage B2 (unfolded leaves hanging down, about 2 cm long, Hallé and Martin 1968) was considered as day 0, following Antas Miguel *et al*. (2007) who showed that before that

stage net photosynthesis was negative. Measurements started four days after this stage as soon as leaves were large enough to be fitted in a 2 × 3 cm leaf chamber. *A* was measured on the same leaf from 4 to 52 days after stage B2. Photosynthetic photon flux (PPF) was set at 1200 μmol m^{-2} s^{-1} which is above the light saturation point for rubber (Nataraja and Jacob 1999). CO_2 concentration was controlled at ambient CO_2 concentration, 360 μmol mol^{-1}. Measurements were recorded about 10–15 min after leaves were enclosed in the leaf chamber *LI-6400* (*Li-Cor*, Lincoln, Nebraska, USA). All leaf gas exchange measurements were made between 09:00 and 15:00 h. During measurements, relative humidity and vapour pressure deficit (VPD) in the leaf chamber ranged from 66–80 % and 1.2–2.5 kPa, respectively. Leaf temperature ranged from 30–35°C.

Experiment 2. Effect of leaf-age class on light-saturated CO_2 assimilation rate and photosynthetic capacity

Plant material: Three widely cultivated rubber tree clones, PB 260, RRIM 600 and RRIT 251 were grown in the field at the Chachoengsao Rubber Research Center (CRRC) (Eastern Thailand, 13° 41' N, 101°04' E, 69 m a.s.l.). Photosynthesis and stomatal conductance were measured in two-year-old trees in October 2006, at the end of the rainy season (Table 1). Rubber trees were planted at a spacing of 7 m between rows and 3 m within rows. Tree height was around 4.5–6 m and canopy width was around 2.5–3.5 m during the measurement period. Trees had 10–15 leaves per whorl, 4–5 whorls per branch and 16–22 branches per tree.

Methodology: As the early stages were studied in pots in experiment 1, the second experiment focused on mature leaves whose precise leaf age was not determined in this experiment. Instead, the whorl rank was taken as an age class surrogate, given that complete leaf shedding in January is followed by new shoot growth in February, beginning with the production of the first whorl (W1).

Whorls of one branch were counted and tagged from the base whorl (W1, oldest) to the top whorl (W5, youngest). A portable scaffolding tower was established and erected around rubber trees during the measurements. The measurements were conducted on 7–8 fully expanded leaves per whorl (4–5 whorls per clone). All leaf gas exchange measurements were made between 09:00 and 15:00 h. During measurements, relative humidity in the leaf chamber was approximately 60–78%, and vapour pressure deficit (VPD) varied from 1.2 to 2.4 kPa, depending on leaf temperature and transpiration rate. Leaf temperature was maintained close to ambient temperature and ranged from 30–34°C. A_{max} and g_s were measured under an ambient CO_2 concentration of 360 μmol mol^{-1} and PPF of 1600 μmol m^{-2} s^{-1}, which is above the light saturation point for field-grown rubber trees (Nataraja and Jacob 1999).

CO_2-response curves were fitted according to the Farquhar *et al*. model (Farquhar *et al*. 1980, Farquhar and von Caemmerer 1982, Harley and Tenhunen 1991) in which net assimilation (*A*) is limited either by the activity of Rubisco at saturating RuBP (A_c) or by RuBP concentration (A_j). Photosynthetic capacity (V_{cmax} and J_{max}) was estimated from the response of *A* to intercellular leaf CO_2 concentration (C_i) (CO_2-response curve or A/C_i curve). The recording of an A/C_i curve was started at an ambient CO_2 concentration of 360 μmol mol^{-1} and a saturating PPF of 1600 μmol m^{-2} s^{-1}. The leaf chamber was equilibrated for at least 15 min in order to reach a steady state. The CO_2 concentration was then decreased stepwise to 250, 200, 150, 100, and 50 μmol mol^{-1} and then increased stepwise from 360 to 600, 800, 1,000; 1,100; 1,200; 1,400 and 1,600 μmol mol^{-1} to obtain A_c and A_j parts of each full curve (RuBP carboxylation and regeneration limited parts of A/C_i curve, respectively). The value of *A* at each concentration was recorded only once *A* and g_s had stabilized. Leaf temperature was

Table 1. Meteorological data at the experimental site (CRRC station, Chachoengsao, Eastern Thailand) in 2006.

Month	Number of rainy days	Rain [mm]	Evaporation pitch [mm]	Mean air temperature [ºC]
January	0	0	162	26.7
February	2	31	159	28.6
March	9	101	173	29.6
April	7	86	157	29.5
May	21	221	156	29.0
June	12	138	151	29.0
July	15	194	154	28.7
August	15	195	149	28.3
September	22	404	115	27.9
October	15	164	140	27.8
November	2	9	143	28.1
December	0	0	156	26.7

Table 2. Primary parameters and their temperature response from Bernacchi *et al*. (2001) used to estimate maximum rate of RuBP carboxylation (V_{cmax}) and maximum rate of electron transport or RuBP regeneration (J_{max}) from A/C_i curves.

Parameter	Units	Values
α (alpha)	mol (electron) mol^{-1}(photon)	0.24
K_c (25°C)	µmol mol^{-1}	404.9
$E_a(K_c)$	J mol^{-1}	79,430
K_o (25°C)	µmol mol^{-1}	278,400
$E_a(K_o)$	J mol^{-1}	36,380
Γ^* (25°C)	µmol mol^{-1}	42.75
$E_a(\tau)$	J mol^{-1}	37,830
O	µmol mol^{-1}	210,000

maintained at a constant ambient level during the measurement cycle. A/C_i curves were fitted by nonlinear least squares regression according to Dreyer *et al*. (2001) using R® software (R® language environment for statistical computing and graphics, *R Foundation for Statistical Computing*, Vienna, Austria), assuming infinite internal conductance (g_i), *i.e* identical CO_2 partial pressure at carboxylation sites and in substomatal cavities. The primary parameter set from Bernacchi *et al*. (2001), suitable for A/C_i curve fitting with infinite g_i, was used. Primary model parameters K_c, K_o, τ and α (apparent quantum yield) are listed with their temperature dependencies in Table 2. The fitting procedure therefore yielded estimates of apparent V_{cmax} and J_{max} (Ethier and Livingston, 2004) (Fig. 1). Details of equations are given in Kositsup *et al*. (2009).

Standardization of apparent V_{cmax} and J_{max} at a reference temperature of 25°C: The temperature varied between measurement days. Hence, apparent values of V_{cmax} and J_{max} had to be standardized to the same reference temperature of 25°C using Eq. 1.

$$P_{25} = P\,\mathrm{e}^{-\left(\frac{E_a}{R}\left(\frac{1}{T_{ref}}-\frac{1}{T}\right)\right)} \quad (1)$$

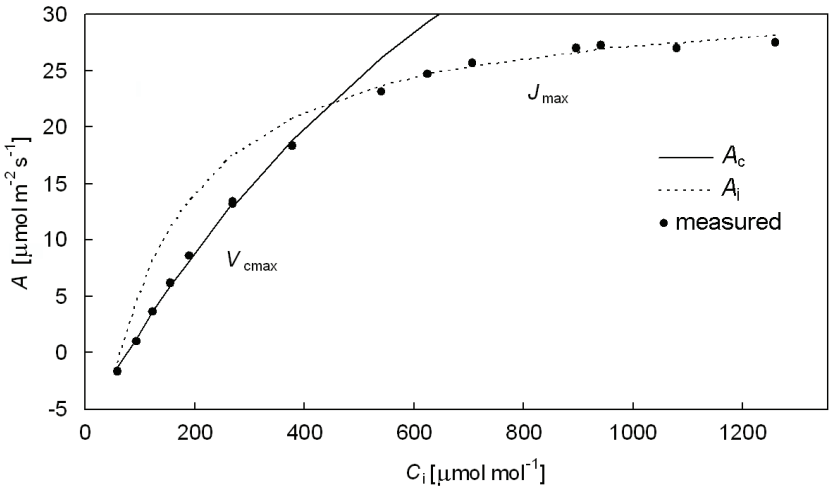

Fig. 1. Example of one A/C_i curve fitting of a 2-year-old potted RRIM 600 clone measured in October 2006. Farquhar's model was fitted to the data of the response of light-saturated CO_2 assimilation rate (A_{max}) to intercellular CO_2 concentration (C_i) in order to estimate V_{cmax} and J_{max}.

where P_{25} is the value of V_{cmax} (or J_{max}) at 25 °C, P is the value of V_{cmax} (or J_{max}) at leaf temperature, T_{ref} is the reference temperature of 25 °C (298.15 K), E_a is the activation energy [J mol^{-1}], R the gas constant (8.314 J K^{-1} mol^{-1}) and T is leaf temperature [K].

The mean activation energy (E_a) of V_{cmax} and J_{max} of rubber clones RRIM 600 and PB 260 (68,514.5 and 50,611.5 J mol^{-1}, respectively) was obtained from the temperature response of photosynthetic capacity (Kositsup *et al*. 2009). Since the temperature response of V_{cmax} and J_{max} has never been studied for clone RRIT 251, the average E_a of RRIM 600 and PB 260 was used to normalize V_{cmax} and J_{max} for all three clones.

Leaf mass per area, nitrogen analysis and chlorophyll content: At the end of the measurement, leaves were collected. Leaf area was measured with a portable leaf area meter, *LI-3100*A (*LI-COR Inc*., Lincoln, NE, USA). Leaves were dried at 70°C for at least 48 h to assess constant dry mass (DM). Leaf samples were ground and nitrogen content (N_m) was analyzed with a nitrogen determination model *FP-528* (*Leco Corporation*, Michigan, USA). Nitrogen per unit leaf area (N_a) was calculated. Photosynthetic nitrogen-use efficiency was expressed as the ratio of A_{max} to N_a.

The chlorophyll content of leaves was measured with a chlorophyll meter (*SPAD-502, Konica Minolta Sensing Inc.*, Osaka, Japan). Six different positions were measured and averaged per leaf.

Data analysis: The effects of clone (df = 2) and whorl rank (df = 3) on dependent variables (leaf area, leaf DM, LMA, SPAD, nitrogen content, A_{max}, J_{max25}, V_{cmax25}, R_D, g_s, A_{max}/N_a) were evaluated by analysis of variance (*ANOVA*) using Statistic Analysis System (*SAS, SAS Institute Inc*., Cary, NC, USA). Seven to eight individual

leaves were used as replicates for each whorl in each clone. *Duncan*'s mean separation test was performed for comparison among clones for a given whorl and among whorls for a given clone. Correlation matrices between dependent variables at the whorl level (n = 12, except for J_{max25} and $V_{cmax25,}$ n = 10) and at the leaf level (n = 92, except J_{max25} n = 48 and V_{cmax25} n = 66) were determined with *XLSTAT 2008* (*Addinsoft SARL*, Paris, France), using Pearson linear correlation coefficients. The relationship between A_{max} and g_s was fitted by an asymptotic function using nonlinear regression analysis (*SAS, SAS Institute Inc*., Cary, NC, USA).

Results

Effect of leaf age on light-saturated CO_2 assimilation rate in potted trees: A_{max} increased with leaf age (Fig. 2) and reached its maximum (about 12 µmol m^{-2} s^{-1}) approximately 35 days after the B2 stage (when leaves were 2 cm long). At that time, leaves were already fully expanded and dark green (data not presented). A_{max} remained high until the end of the experiment, 52 days after the B2 stage.

Effect of leaf-age class on leaf characteristics, light-saturated CO_2 assimilation rate and photosynthetic capacity

Leaf morphology and nitrogen content: In October 2006, canopies of rubber tree clones RRIM 600, PB 260 and RRIT 251 comprised 5, 4 and 4 leaf-age classes (whorls), respectively. Leaves in the older whorls (W1 and W2) had smaller area and less DM than the younger ones (W3 to W5). Leaf mass per area (LMA) decreased significantly with whorl rank in PB 260, but not clearly in RRIM 600 and RRIT 251 (Fig. 3). Leaf greenness as indicated by the SPAD was lower in RRIT 251 than in the two other clones. N_m increased with whorl rank (decreased with leaf-age class) for each clone except in W5 in RRIM 600, whereas N_a showed no clear dependence on whorl rank. The range in nitrogen content (N_a and N_m) was low. When mean data per whorl were compared and all clones pooled together, there was no significant correlation between SPAD and nitrogen content, either N_a or N_m (Table 3). In individual clones, there was a weak correlation between SPAD and N_a in PB 260 (P<0.10; R^2 = 0.13) and in RRIM 600 (P<0.01; R^2 = 0.21). When all individual leaves were considered (Table 4), SPAD and N_a were correlated, but with a low R^2.

Leaf gas exchanges: A_{max} per whorl ranged from 2.1–13.9 µmol m^{-2} s^{-1} (Fig. 4). A_{max} decreased with leaf-age class (increased with whorl rank) and was significantly different (P<0.05) among clones only in W3 (Table 5). A_{max} differed significantly between each whorl (W1 to W4) in PB 260, whereas there was no difference among youngest whorls in RRIM 600 (W3–W5) and in RRIT 251 (W3–W4). Nevertheless, there was a decreasing trend in A_{max} with leaf-age class for all three clones. A_{max} of leaves of the oldest age class (W1) ranged from approximately 15% (RRIT 251) to 32% (RRIM 600) of that of leaves in the youngest age class (W4). Nitrogen-use efficiency (A_{max}/N_a) correlated with leaf-age class in the same way as A_{max} except in W5 in RRIM 600. A_{max} did not correlate with N_a at whorl level (Table 3), although the correlation was high but with a low R^2 at a leaf level (Table 4). In PB 260 and RRIM 600, dark respiration (R_D) was much higher in the youngest leaf-age class (W4 in PB 260 and W5 in RRIM 600) than in the other classes, whereas in RRIT 251, there was a steady decrease of R_D from W4 to W1.

Photosynthetic capacity: Parameters of the biochemical model of leaf photosynthesis, *i.e.* J_{max} and V_{cmax}, could not be estimated for the oldest leaf-age class (W1) in all three clones, as g_s was too low, less than 0.04 mol m^{-2} s^{-1} and A/C_i curves obtained from these leaves were not reliable (Fig. 4). Mean J_{max25} per whorl ranged from 73.4–115.1 µmol m^{-2} s^{-1} and mean V_{cmax25} ranged from 49.4 to 65.9 µmol m^{-2} s^{-1}.

Whorl rank (leaf-age class) also had a significant effect (P<0.001) on photosynthetic capacity. J_{max25} and V_{cmax25} decreased significantly with leaf-age class (Fig. 4), except in some cases where the photosynthetic capacity of the youngest whorl was lower than that of the previous one. J_{max25} of the oldest measured leaf class (W2) was 62% (PB 260) to 79% (RRIM 600) of the maximum values (W3 or W4). V_{cmax25} of oldest measured leaves (W2) was 83% (RRIM 600) to 88% (RRIT 251) of maximum values (W3 or W4). PB 260 had lower V_{cmax25} and J_{max25} than RRIM 600, and RRIT 251 had intermediate values. When all clones were pooled, A_{max} was significantly correlated to V_{cmax25} and J_{max25}, but R^2 values were low (Table 3) when individual leaves were consi dered. However, the coefficient of determination was higher when leaves were pooled by whorl, particularly for J_{max25} (R^2 = 0.81, Table 3). V_{cmax25} and J_{max25} did not correlate with nitrogen content, either at leaf level or at the whorl one (Tables 3, 4).

g_s decreased strongly with leaf-age class (increased with whorl rank), except in W5 of RRIM 600 (Fig. 4). The very low g_s in W1 (oldest leaves) did not allow estimation of J_{max} and V_{cmax}. In W1 and W2, PB 260 had a significantly lower g_s than RRIT 251, whereas RRIM 600 was medium (Table 5). In W3 and W4, there was no significant difference in g_s between clones. At the leaf level, g_s was correlated with all other parameters (A_{max}, R_D, $V_{cmax25,}$ $J_{max25,}$ N_m, N_a, and SPAD) (Table 4). At whorl level, g_s was correlated with all parameters except for N_a

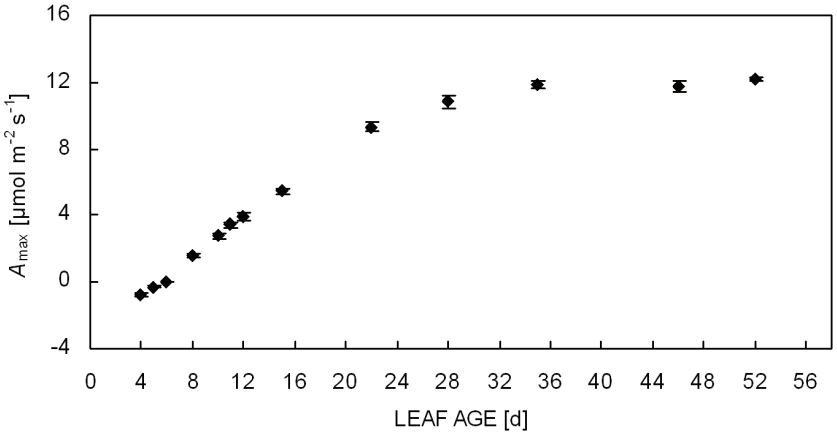

Fig. 2. Mean maximum rate of light-saturated CO_2 assimilation (A_{max}) as a function of leaf age of 2-month-old potted rubber, clone RRIM 600. Each value represents the average ± SE (n = 6).

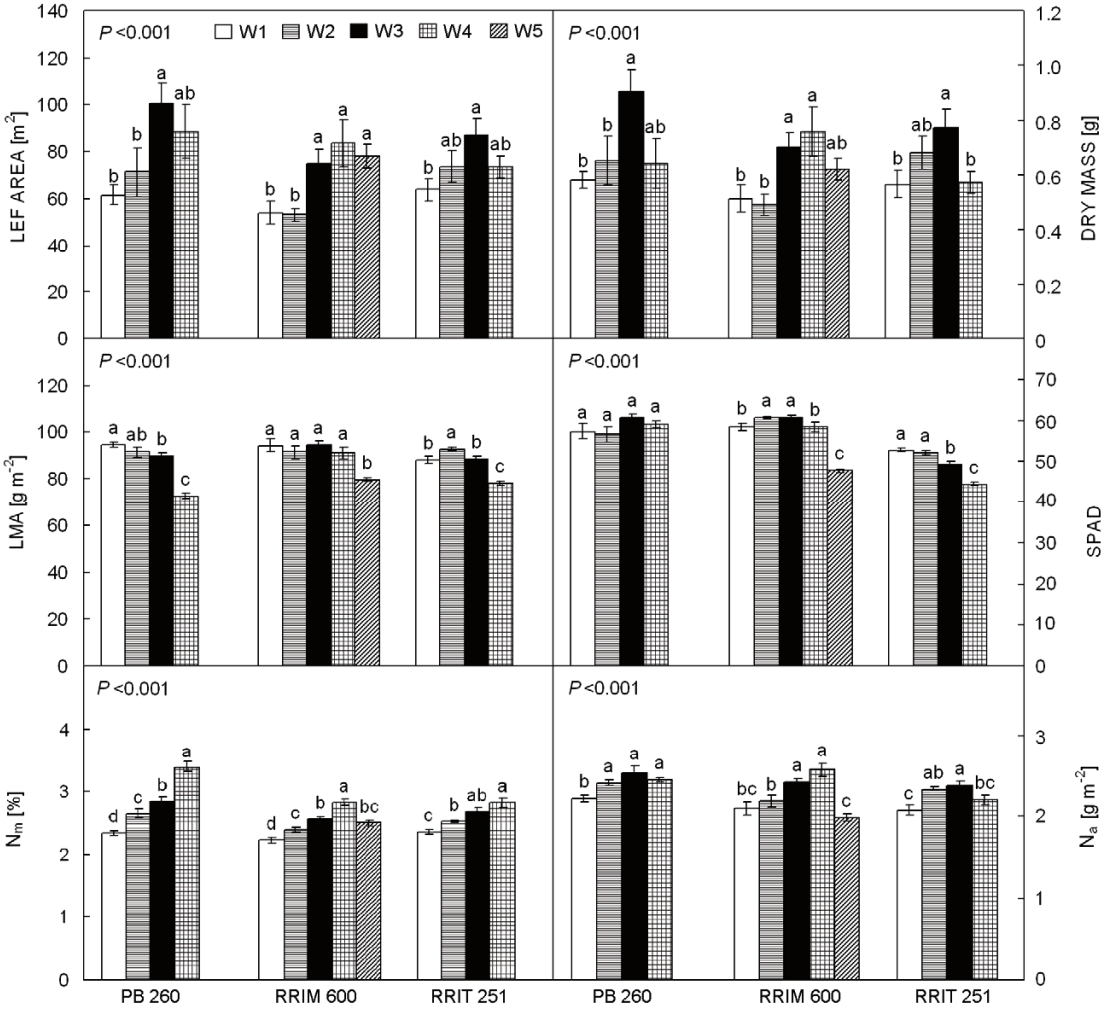

Fig. 3. Effect of leaf-age class, or whorl position (oldest whorl W1 to youngest whorl W5), on leaf characteristics of 2-year-old rubber trees (3 clones, PB 260, RRIM 600 and RRIT 251): leaf area (LA), dry mass (DM), leaf mass per area (LMA), SPAD, nitrogen per unit mass (N_m), nitrogen per unit area (N_a). Each value represents the average ± SE of 7–8 leaves per flush. Different letters indicate values significantly different between whorls of the same clone (*Duncan* P<0.05).

and SPAD (Table 3). Fig. 5 shows that when all clones are pooled, the relationship between A_{max} and g_s could be modeled by an asymptotic function (R^2 = 0.85 at the leaf level, R^2 = 0.94 at the whorl level, not shown) with a maximum value of about 16 µmol m^{-2} s^{-1}. The relationship between A_{max} and g_s was similar in all 3 clones (Fig. 5).

Discussion

Evolution of net photosynthesis with age in potted trees: In our experimental conditions, the rubber leaves were completely expanded 13 days after stage B2 (leaf hanging down, 2 cm long, Sangsing *et al*. 2004). Maximum A_{max} was obtained about 22 days after the complete leaf expansion. Such results were in accordance with Samsuddin and Impens (1979b) and Antas Miguel *et al*. (2007). A decline following a peak reached 34 days after leaf emergence was reported by Samsuddin and Impens (1979b) in some clones, but not in RRIM 600. PB 260 and RRIT 251 were not studied by these authors.

Table 3. Correlation matrix (Pearson coefficient) between variables at leaf-age class (whorl) level. Light-saturated CO_2 assimilation rate (A_{max}), dark respiration (R_D), stomatal conductance (g_s), maximum rate of RuBP carboxylation (V_{cmax25}), maximum rate of electron transport or RuBP regeneration (J_{max25}), nitrogen content per unit mass (N_m), nitrogen content per unit area (N_a) and chlorophyll content (SPAD). Bold numbers indicate significant correlations. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Variables	R_D	g_s	V_{cmax25}	J_{max25}	N_m [%]	N_a [g m^{-2}]	SPAD
A_{max}	**0.73*****	**0.87*****	**0.46***	**0.81*****	**0.47****	0.23	0.06
R_D		**0.63****	0.15	0.25	0.30	0.01	0.20
g_s			**0.45***	**0.80****	**0.39***	0.11	0.17
V_{cmax25}				**0.73****	0.04	0.01	0.01
J_{max25}					0.11	0.00	0.05
N_m [%]						**0.41***	0.00
N_a [g m^{-2}]							0.21

Table 4. Correlation matrix (Pearson coefficient) between variables at leaf level. Light-saturated CO_2 assimilation rate (A_{max}), dark respiration (R_D), stomatal conductance (g_s), maximum rate of RuBP carboxylation (V_{cmax25}), maximum rate of electron transport or RuBP regeneration (J_{max25}), nitrogen content per unit mass (N_m), nitrogen content per unit area (N_a) and chlorophyll content (SPAD). Bold numbers indicate signficant correlations. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Variables	R_D	g_s	V_{cmax25}	J_{max25}	N_m [%]	N_a [g m^{-2}]	SPAD
A_{max}	**0.37*****	**0.68*****	**0.14****	**0.28*****	**0.36*****	**0.15*****	**0.05***
R_D		**0.21*****	**0.15****	**0.12***	**0.16*****	0.02	**0.11****
g_s			**0.07***	**0.25*****	**0.27*****	**0.06***	**0.11****
V_{cmax25}				**0.44*****	0.01	0.05	0.00
J_{max25}					0.03	0.00	0.04
N_m [%]						**0.38*****	0.00
N_a [g m^{-2}]							**0.13*****

Significance of leaf whorl rank: As the number of whorls varied between clones, it was difficult to estimate the age of each class precisely and not possible to determine the age of each leaf within a class. In RRIM 600, this estimation was easier than in other clones, as leaves of the last whorls were fully expanded but not completely mature as indicated by low DM, LMA, N content, SPAD, A_{max} photosynthetic parameters (J_{max}, V_{cmax}), and high R_D. As rubber LMA increases during 10–15 days after complete leaf expansion (Thaler and Pagès 1996) which itself lasts about 13–15 days (Hallé and Martin 1968, Sangsing *et al*. 2004), we can estimate that these leaves were 20–30 days old. The remaining 200-day interval between refoliation and the establishment of the last whorls implies a whorl-development duration of about 50 days. This duration is in accordance with measurements by Hallé and Martin (1968).

Age of leaf classes could not be determined with such precision in PB 260 and RRIT 251. More accurate notations of phenology are required to know the exact age of the different whorls grown within one year. Moreover, the oldest and the lowest whorls (W1 and W2) had a lower final leaf area and mass than W3 and W4. This showed that differences between whorls could not be considered as being related to leaf age only. There could be differences in morphology and physiology according to whorl position. Due to their different location within the canopy, low or high whorls could also have different PPF environment, interfering with the effect of leaf age, particularly on nitrogen content (Le Roux *et al*. 2001). However, because of the spacing (3 × 7 m) and the size of the trees when the experiment took place, PPF environment was not likely to be different between leaves, which could all be considered as sun-leaves. This could explain the low variability in nitrogen content among whorls.

Although further studies are necessary to distinguish leaf-age effect from the whorl-location one, whorl rank proved to be an appropriate parameter to classify leaves

Table 5. *ANOVA* of clonal effects on light-saturated CO_2 assimilation rate (A_{max}), stomatal conductance (g_s), maximum rate of RuBP carboxylation (V_{cmax25}) and maximum rate of electron transport or RuBP regeneration (J_{max25}) in individual leaves of each leaf-age class (whorl). W1 is the oldest leaf-age class and W4 is the youngest. Values followed by different letters differ significantly (*Duncan*, $P<0.05$). a*: PB 260 differed from RRIT 251 but not from RRIM 600. V_{cmax} and J_{max} could not be estimated in the oldest class (W1).

Whorl		A_{max}	g_s	V_{cmax25}	J_{max25}
W1	P>F	ns	0.045		
	RRIT 251	2.1	0.053 a		
	RRIM 600	4.1	0.050 a		
	PB 260	3.2	0.029 a*		
W2	P>F	Ns	0.048	ns	ns
	RRIT 251	10.4	0.139 a	50.5	88.7
	RRIM 600	8.3	0.101 ab	56.0	93.8
	PB 260	7.1	0.058 b	49.5	70.2
W3	P>F	0.040	ns	0.035	0.017
	RRIT 251	12.8 a	0.218	62.2 a	118.6 a
	RRIM 600	12.2 ab	0.188	57.9 b	109.8 a
	PB 260	10.0 b	0.110	57.5 b	89.7 b
W4	P>F	ns	ns	0.002	ns
	RRIT 251	13.9	0.281	67.1 a	111.0
	RRIM 600	12.7	0.220	58.9 b	118.3
	PB 260	12.9	0.200	57.5 b	110.8

in the purpose of modelling canopy photosynthesis. This is an easy-to-access parameter integrating both age and architectural effects.

Effect of whorl rank on photosynthesis of 2-year-old field-grown rubber trees: A_{max} of whorls W3 to W5 was in the same range as A_{max} of young leaves in the pot experiments and in previously reported experiments. Among twelve clones, Nataraja and Jacob (1999) found A_{max} to vary between 11 and 15 µmol m^{-2} s^{-1}. A_{max} in RRIM 600 was similar to results of Samsuddin *et al*. (1987) and Ceulemans *et al*. (1984). PB 260 was found to have a higher A_{max} (24.8 µmol m^{-2} s^{-1}) by Ceulemans *et al*. (1984), but not by Samsuddin *et al*. (1987) (10.3 µmol m^{-2} s^{-1}). RRIT 251, a clone recently selected in Thailand, was not included in previous clonal comparisons of photosynthesis. Therefore we confirmed the clonal effect reported in previous studies, but we also showed that there was a larger variability in A_{max} between whorls of the same clone than between clones for the same whorl rank. The strong decrease in A_{max} with leaf age was not reported in studies by Samsuddin and Impens (1979b) and Antas Miguel *et al*. (2007) because these authors did not study leaves older than 100 days.

This study reports the first estimation of two major parameters determining the biochemical capacities of the photosynthetic apparatus, the maximum rate of carboxylation (V_{cmax}) and the maximum rate of electron transport (J_{max}) in field-grown clones of rubber trees. This is an important step for modelling canopy photosynthesis in this major tree crop. Mean J_{max} and V_{cmax} were in the upper part of the range recorded in fourteen tropical rain forest species (Costes *et al*. 2005). The much lower decrease in J_{max} and V_{cmax} with leaf age, as compared to A_{max}, showed that photosynthetic capacity was not the major factor affecting A_{max} in old leaves. This was confirmed by the low coefficient of determination of the correlation at leaf level between A_{max} and J_{max} and particularly A_{max} and V_{cmax}. V_{cmax} and nitrogen content both showed little variation, possibly indicating that Rubisco concentrations are stable and allow the maintenance of a high RuBP carboxylation capacity. However this interpretation was limited by the impossibility to estimate J_{max} and V_{cmax} in the oldest leaves (W1) which had the lowest A_{max}. J_{max} and V_{cmax} did not correlate with nitrogen content, contrary to previous reports on other species (Harley *et al*. 1992, Le Roux *et al*. 1999, Niinemets *et al*. 1999). However, a large part of the variation in nitrogen content within the canopy reflects adaptation to differences in irradiance (Boardman 1977, Niinemets *et al*. 1999, Frak *et al*. 2001). In our conditions, irradiance levels were relatively homogeneous, as trees were still isolated and exhibited little or no self-shading, possibly explaining the low range in nitrogen content. In older rubber plantations with a closed canopy, we can expect larger changes in nitrogen content between shaded and sunlit leaves, and also more changes in photosynthetic parameters (Senevirathna *et al*. 2003, Vincent 2006). We observed that stomatal conductance was highly variable and well correlated with A_{max}. These results were obtained in wet conditions (Table 1), meaning that climatic limitations on g_s are unlikely to explain the correlation. Samsuddin and Impens (1979a) also found that stomatal conductance might be important to explain

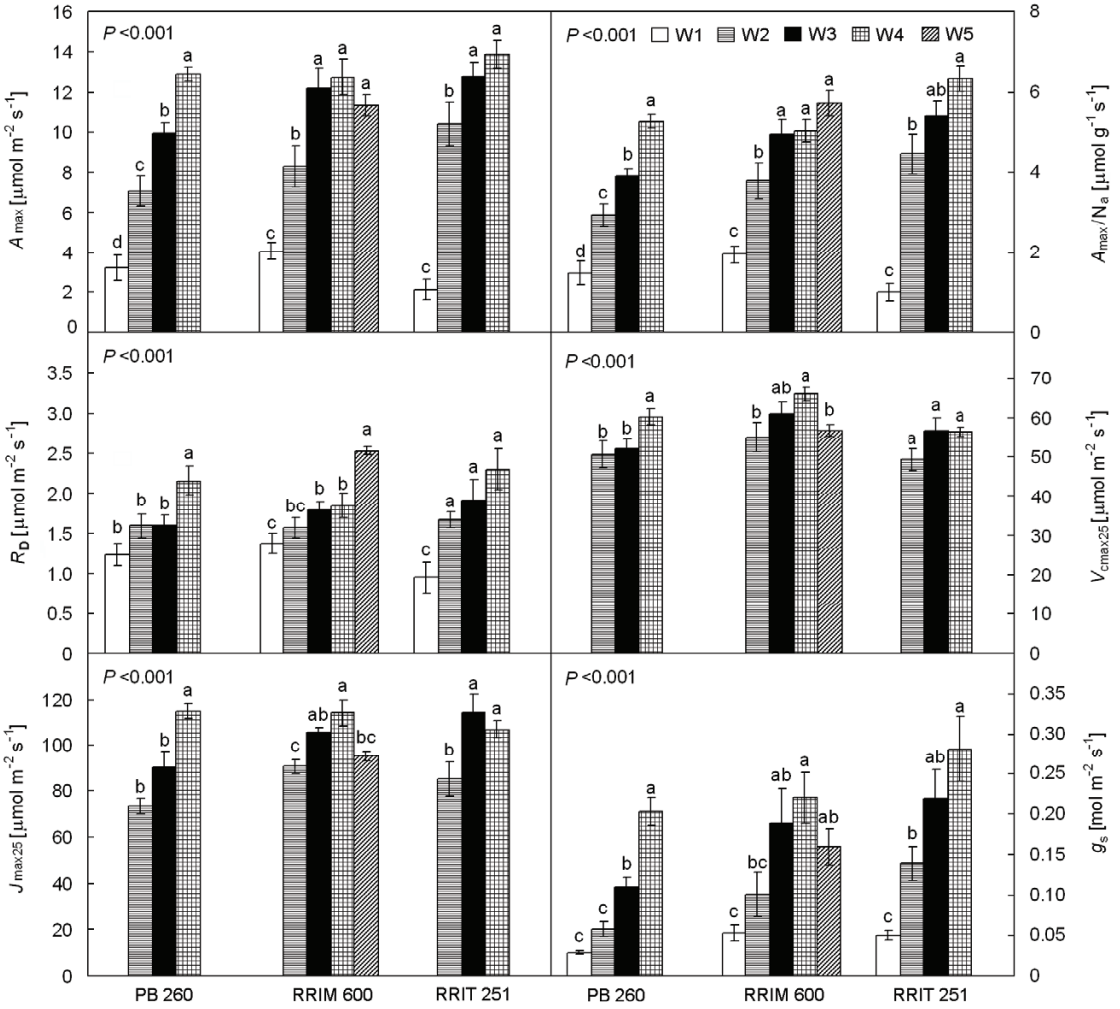

Fig. 4. Effect of leaf-age class, or whorl rank (oldest whorl W1 to youngest whorl W5), on leaf gas exchange of 2-year-old rubber trees (3 clones, PB 260, RRIM 600 and RRIT 251): light-saturated CO_2 assimilation rate (A_{max}), photosynthetic nitrogen-use efficiency (A_{max}/N_a), dark respiration (R_D), maximum rate of carboxylation (V_{cmax}), maximum rate of electron transport (J_{max}) and stomatal conductance (g_s). Each value represents the average ± SE of 7 leaves per flush. Different letters indicate values that differ significantly between whorls of the same clone (*Duncan* P<0.05).

differences in photosynthesis between rubber clones, and Sobhana *et al*. (1996) observed a correlation between g_s and A_{max} in rubber seedlings. However, the co-evolution of A_{max} and g_s with leaf age in rubber trees has not been described before. The decrease in g_s we observed with leaf aging has been reported in other species (Jordan *et al*. 1975, Field and Mooney 1986, Han *et al*. 2008). However, the phenology of tropical species differs from that of temperate species, particularly leaf lifespan is often longer in tropical species (Vincent 2006). But few data are available on the latter. Kitajima *et al*. (2002) showed that g_s decreased with age (up to 90 days) in only one of the 2 pioneer tropical tree species they studied. The strong decrease in both A_{max} and g_s in older whorls could be due to the onset of leaf senescence. However LMA did not vary between mature leaves, indicating that their morphology was likely to be similar and that they were neither thickening, nor decaying. Evolution of SPAD also indicated no decrease in leaf greenness in mature whorls. The stronger decrease with leaf age in N_m than N_a indicated that there was no or little exportation of N from old leaves, but rather dilution (accumulation of non-N chemicals), likely starch, known to accumulate as leaves mature (Belesky and Hill 1997, Patakas and Noitsakis 2001, Szadel *et al*. 2003). Such an accumulation of photosynthetic products may be a direct or indirect cause of the limitation of assimilation in older leaves, as sink regulation of photosynthesis has been demonstrated (review by Paul and Foyer 2001). However, such regulation is usually considered on a whole plant level, or when branches are artificially isolated (Urban *et al.* 2004). It is not likely that the different whorls of a branch would be differentially affected by changes in carbon demand. Moreover, regulation of photosynthesis by sink activity is mainly mediated by changes in nitrogen allocation, which were not observed in our study.

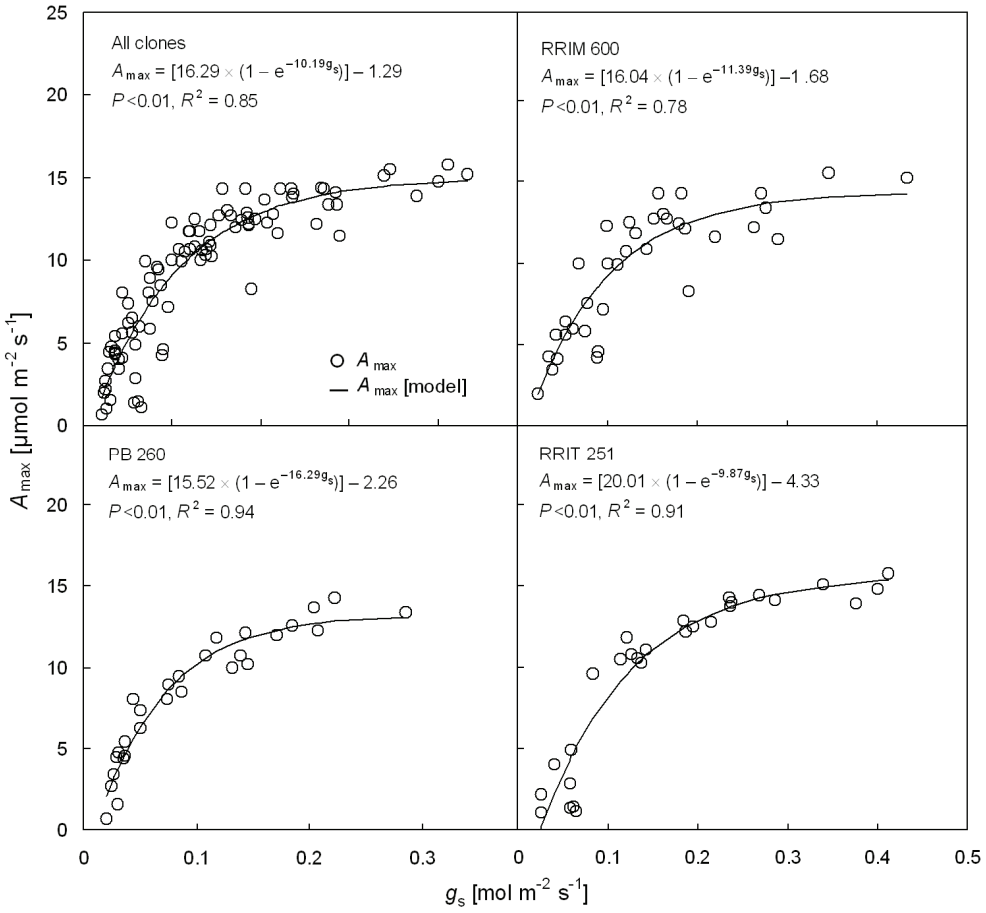

Fig. 5. Relationship between stomatal conductance (g_s) and light-saturated CO_2 assimilation rate (A_{max}) of 2-year-old rubber trees. Dots represent observed data and the line represents an asymptotic function fitted to the observations. All clones pooled together (*top left*), clone RRIM 600 (*top right*), clone PB 260 (*bottom left*) and clone RRIT 251 (*bottom right*).

In many models devoted to canopy photosynthesis, the Jarvis (1976) model of stomatal conductance is coupled with Farquhar's model (1980) of leaf photosynthesis, and g_s is indirectly used instead of C_i in the equations describing the carboxylation rate limited by Rubisco (W_c) and the carboxylation rate limited by RUBP regeneration (W_j) (Wang and Jarvis 1993). However, maybe because the Farquhar's model sounds more biochemically grounded, whereas the Jarvis' model is purely empirical, the latter has received less attention. Our results showed that in field-grown rubber trees, modelling stomatal conductance is of first importance in order to model canopy photosynthesis.

Conclusions: Whorls were revealed as a practical variable to classify leaves in gas exchange studies on field-grown rubber trees. This could be expanded to many tropical species having the same periodic growth patterns.

Light-saturated CO_2 assimilation rate (A_{max}) varied much more between whorls of the same clone than between clones. It may be more important to take into account the whorl effect than the clone effect in studies of photosynthesis in field-grown rubber trees.

The decrease in photosynthetic capacity, as indicated by J_{max} and V_{cmax}, could not explain the strong decrease in A_{max} with leaf age.

Stomatal conductance was the most important factor explaining changes in A_{max} and attempts to model canopy photosynthesis of the rubber tree should focus on this function.

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